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File: USPT

Dec 1, 1998

US-PAT-NO: 5843741

DOCUMENT-IDENTIFIER: US 5843741 A

TITLE: Method for altering the differentiation of anchorage dependent cells on an electrically conducting polymer ..

DATE-ISSUED: December 1, 1998

### INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wong; Joyce Y.	Cambridge	MA		
Ingber; Donald E.	Boston	MA		
Langer; Robert S.	Newton	MA		

US-CL-CURRENT: 435/173.8

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2. Document ID: US 5134070 A

L7: Entry 2 of 2

File: USPT

Jul 28, 1992

US-PAT-NO: 5134070

DOCUMENT-IDENTIFIER: US 5134070 A

TITLE: Method and device for cell cultivation on electrodes

DATE-ISSUED: July 28, 1992

### INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 435/173.6; 204/406, 435/289.1, 435/298.1, 435/305.4, 435/461, 435/817

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Drawing Description Text (3):

FIG. 2 is a graph of DNA synthesis in cells cultured in serum-containing medium on various FN-coated substrata: ITO (-), ITO (+), polypyrrole (-), polypyrrole (+), and PD (-), in the absence (-) or presence (+) of an applied electrical potential (-0.25 V). Data are presented as mean.+-.standard error of the mean. ITO stands for indium tin oxide electrode, PPY for polypyrrole, PD for Petri dish. Cell viability was greater than 90% under all conditions.

Detailed Description Text (69):

(a) Optically-transparent thin films of polypyrrole were synthesized which were suitable for viewing under the inverted phase-contrast light microscope. The electrochemical method was used, since it allows easy control of film thickness. Thin films of polypyrrole were synthesized potentiostatically at 1.1 V (versus saturated calomel electrode) on an optically-transparent indium tin oxide (ITO) from a solution containing 0.1M pyrrole (purified on an activated alumina column) and 0.1M tosylate in acetonitrile with 0.05 v % ultrapure water. Diaz, A. F.; Kanazawa, K. K. Extended Linear Chain Compounds; Plenum Press: New York, 1983, pp. 417-441. In addition, the solution was degassed with Ar before each film deposition. Film thicknesses were on the order of 0.1 .mu.m. Wernet, W., et al., Makromol. Chem., 188, 1465-1475 (1987).

Detailed Description Text (74):

In order to further study the effect of reduction of the polypyrrole substrate on cell shape, the effect was studied of applying a reduction potential to FN-coated polypyrrole surfaces on which cells had been first allowed to attach and spread. After application of -0.25 V (vs. Ag/AgCl) for 1 hour, the pseudopods of the cells began to retract, and the cells became rounded. Cells were not being killed since the viability of the rounded cells was 84.+-.5% (compared to 91.+-.2% for spread cells on FN-coated petri dishes) as determined by the Live/Dead.RTM. Viability/Cytotoxicity assay (Molecular Probes Inc., Eugene, Oreg. 97402). As shown by the cyclic voltammogram during reduction, the cells are exposed only to a current density of 20 .mu.A/cin, and a current density of a few mA/cm.sup.2 was shown not to affect cell attachment, spreading and growth of fibroblasts. Giaever, I.; Keese, C. R. Proc. Natl. Acad. Sci., USA, 81, 3761-3764 (1984). In addition, when the same experiment was repeated on FN-coated gold or indium tin oxide (ITO) surfaces, cells did not round up after 1 hour application of -0.25 V (vs. Ag/AgCl). Thus, this effect most likely is not due to an electrical field. Furthermore, since (1) the conductivity of electrochemically reduced polypyrrole films are on the order of 10.sup.-6 Scm.sup.-1 (Qian, R.; Qiu, J. Polym. J., 19, 157-182 (1987)), compared to approximately 1 Scm.sup.-1 for cell culture medium, and (2) the cells are not a confluent monolayer, the current generated is directed towards reducing the polymer film and not the cells.

Detailed Description Text (76):

(b) Synthesis of Polypyrrole. Pyrrole (Kodak, Rochester, N.Y.) was purified by passage through an activated alumina column until it became colorless. Electrochemical synthesis of polypyrrole (Diaz, A. P., et al., J. Chem. Soc. Chem. Commun. 14, 635-636 (1979)) was carried out in an electrochemical cell containing an optically transparent indium tin oxide anode (40 ohms per square; Delta Technologies, Stillwater, Minn.), a platinum mesh counter-electrode, and a saturated calomel reference electrode (SCE). The electrodeposition solution contained 0.1M tetraethylammonium p-toluenesulfonate (Alfa Products, Ward Hill, Mass.) in acetonitrile with 0.5% (vol/vol) ultrapure water (Milli-Q Reagent Water System; Millipore). Films were made potentiostatically at 1.1 V (versus SCE; Pine Instruments AFRDE4 bipotentiostat; Linseis x-y

recorder) until about 100 mC/cm<sup>2</sup> was passed. J. Y. Wong, et al., PNAS, 91:3201-3204 (1994).

Detailed Description Text (81):

The electrochemical synthesis resulted in formation of uniform films of polypyrrole on indium tin oxide-coated glass substrates. Film thicknesses were estimated from the amount of charge passed during electrodeposition (Wernet, W. & Wegner, W., Makromol. Chem. 188, 1465-1475 (1987)) and were about 0.1 .mu.m. Polypyrrole obtained via electrochemical synthesis was in its oxidized state as a polycation with dopant anions to balance the charge (FIG. 1) and was able to be cycled between its charged and neutral forms electrochemically in culture medium, as shown by a cyclic voltammogram of polypyrrole in serum-free culture medium, at a scan rate of 50 mV/sec. The oxidation state of polypyrrole was monitored by UV/visible spectroscopy in its native oxidized state under no potential, reduced by application of -0.25 V, or -0.5 V. A broad peak near 800 nm associated with the bipolarons (Scott, J. C., et al., Synth. Met. 9, 165-172 (1984)) was present in the oxidized polymer. Application of -0.5 V switched polypyrrole to its neutral state, as indicated by the disappearance of the peak near 800 nm and the appearance of a separate peak near 370 nm. This latter peak has been previously shown to be characteristic of the neutral polymer (Genies, E. M., et al., J. Electroanal. Chem. 191, 111-126 (1985)). At -0.25 V, the spectrum fell between those of the oxidized and neutral states. The polymer spectra stabilized within 30 seconds after the potential was applied. When the reduction potential was removed, the neutral polymer reverted completely to its oxidized state within 30 seconds. This phenomenon has been observed by others as well (Li, Y., et al., Synth. Met. 28, C127-C132 (1989)).

Detailed Description Text (83):

Cell shape and growth have been shown to be tightly coupled in many anchorage-dependent cells (Folkman, J., et al., Nature (London) 273, 345-349 (1978); Ben-Ze'ev, A., et al., Cell 21, 365-372 (1980); Ingber, D. E., Proc. Natl. Acad. Sci. USA 87, 3579-3583 (1990); Mooney, D., et al., J. Cell. Physiol. 161, 497-505 (1992); Ingber, D. E., et al., J. Cell Biol. 109, 317-330 (1989)). Similarly, these experiments show that cell retraction induced by applying an intermediate electrical potential (-0.25 V) to FN-polypyrrole provided control over cell cycle progression (FIG. 2). Approximately 75% of cells cultured in serum-containing medium on FN-coated Petri dishes, indium tin oxide, or oxidized polypyrrole spread normally and entered S phase synchronously 15-20 hours after plating. Applying -0.25 V to indium tin oxide had little effect on either cell growth or form. In contrast, few cells (<2%) synthesized DNA when the same electrical potential was used to switch the FN-polypyrrole to its neutral state and promote cell retraction. Similar results were obtained when chemically defined, serum-free medium was utilized, except that labeling indices were slightly lower (date not shown). These effects were not due to cell death, since >90% of the cells remained viable on the neutral FN-polypyrrole, as determined by quantitating incorporation of the vital dye calcein acetoxymethylester.

Detailed Description Text (87):

Electrochemical synthesis of polypyrrole was carried out in an electrochemical cell containing an optically-transparent indium tin oxide (ITO) anode (Delta Technologies, Stillwater, Minn.), platinum mesh counter electrode, and a pseudo Ag wire reference electrode. Electrodeposition (AFRDE4 bipotentiostat, Pine Instruments Co., Grove City, Pa. 16127) was performed in a solution of purified pyrrole (0.1M), tetraethylammonium-p-toluene sulfonate (0.1M, Alfa Products, Ward Hill, Mass. 01835), highly pure water (0.5 v/v %) and acetonitrile. Polypyrrole films were made potentiostatically at 0.8 V (vs. Ag wire) until about 150 mC/cm<sup>2</sup> was passed, which corresponded to a thickness 1 .mu.m.

Detailed Description Paragraph Table (1):

TABLE I		Conductivity and Contact Angle Results
Conductivity	Contact angle (.OMEGA.-cm) <sup>-1</sup> (.degree.)	PD sup. 10. sup.-15 81 .+- .6 TC sup. 10. sup.-15 55 .+- .5 PPY/PD 30 71 .+- .5 PPY/TC 30 71 .+- .4 PPY/ITO 1 73 .+- .5 ITO 800 55 .+- .6

PD Tissue culture grade polystyrene dishes  
Bacteriologic grade polystyrene dishes  
PPY/PD Polypyrrole deposited onto PD  
PPY/TC Polypyrrole deposited onto optically transparent indium tin oxide anode  
ITO optically transparent indium tin oxide anode

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Detailed Description Text (9):

Since conductive and semi-conductive thin films tend to suffer from high in the plane resistance, an alternate method is shown in FIG. 2a where there is deposited a transparent, thin film 24 of metal, preferentially one of the noble metal family, e.g. platinum or gold, of approximately 50 to 200, e.g. 100, Angstroms thickness onto the base substrate 1 forming a radial distribution electrode upon which a thin layer of coating 2 having a thickness of 0.1 to 5 microns of suitable material for cell adhesion, preferably of tin and/or indium oxides, is deposited. This arrangement gives rise to an overall reduction in resistance. In a further embodiment, the preferably noble metal may be replaced by a further semi-conductor having better conductivity properties, but unusable as a cell cultivation surface for reasons such as toxicity, electro-chemical stability, etc. This second semi-conductor would, as the noble metal film, have deposited on its' surface, semi-conductor materials conducive to cell growth such as tin oxide or pin oxide with indium, etc. If a transparent thin-film layer of metal, or a layer of a more conductive transparent thin-film semi-conductor is applied firstly to the substrate, the coating may be in the thinner range stated above.

Detailed Description Text (12):

The exact material/materials used for the coating/coatings will depend upon such parameters as transparency, resistivity, chemical stability, mechanical stability, biological inertness, cost, preferred methods of application, etc. However, preferred materials for forming the coating 2 are tin oxide ( $\text{SnO}_{\text{sub.}2}$ ) and indium oxide and various combinations of the two, (the ITO family) and various combinations of the two doped with other materials. Other materials suitable as transparent thin film conductors and which may be employed for forming the coating 2 or layer 2a include tin oxide doped with either fluorine or antimony and indium oxide doped cadmium oxide, cadmium stannate, zinc oxide, zinc cadmium sulfite, and titanium nitride (TiN). Materials currently showing promise for use as transparent electrodes and which may also be contemplated for forming the coating 2 or layer 2a are: rubidium silver iodide ( $\text{RbAg}_{\text{sub.}4}\text{I}_{\text{sub.}5}$ ), dieuropium trioxide, lanthanum hexaboride, rhenium trioxide, and divanadium pentaboride.

## CLAIMS:

5. Apparatus as claimed in claim 4, wherein the semiconductor material is selected from the group consisting of tin oxide, indium oxide and mixture thereof.

6. Apparatus as claimed in claim 4, wherein the conductive coating is selected from the group consisting of stannic oxide doped with fluorine or antimony, indium oxide doped with cadmium oxide, cadmium stannate, zinc oxide, zinc cadmium sulfite and titaneium nitride.